### **Refine Search**



#### Search Results -

Term	Documents
SUPERSCRIPT	6130
SUPERSCRIPTS	1425
(1 AND SUPERSCRIPT).USPT,EPAB,JPAB,DWPI.	0
(L1 AND SUPERSCRIPT ).USPT,EPAB,JPAB,DWPI.	0

US Pre-Grant Publication Full-Text Database
US Patents Full-Text Database
US OCR Full-Text Database
EPO Abstracts Database
JPO Abstracts Database
Derwent World Patents Index
IBM Technical Disclosure Bulletins

Search:

L2

Database:

Exercises (	Refine Search

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### **Search History**

DATE: Friday, May 21, 2004 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB = USPT,	EPAB,JPAB,DWPI; PLUR=	YES; OP=ADJ	-
<u>L2</u>	L1 and superscript	(	<u>L2</u>
<u>L1</u>	5968784.pn.	2	<u>L1</u>

Recall Text

**END OF SEARCH HISTORY** 

# Freeform Search

Database:	US Pre-Grant Publication Full-Text Database US Patents Full-Text Database US OCR Full-Text Database EPO Abstracts Database JPO Abstracts Database Derwent World Patents Index	1				
	IBM Technical Disclosure Bulletins					
Term:	L11 and (mak\$3 cDNA or produc\$3 cDNA or prepar\$3 cDNA or sysnthesiz\$3 cDNA)					
Display:	Documents in <u>Display Format</u> : Starting with Number 1					
Generate: C Hit List © Hit Count C Side by Side C Image						
Search Clear Interrupt						

## DATE: Friday, May 21, 2004 Printable Copy Create Case

Set Name side by side	Query	<u>Hit</u> <u>Count</u>	Set Name result set		
DB=USPT,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ					
<u>L12</u>	L11 and (mak\$3 cDNA or produc\$3 cDNA or prepar\$3 cDNA or sysnthesiz\$3 cDNA)	2	<u>L12</u>		
<u>L11</u>	SuperScript same (benefit\$1 or advantag\$4)	71	<u>L11</u>		
<u>L10</u>	SuperScript near5 (benefit\$1 or advantag\$4)	0	<u>L10</u>		
<u>L9</u>	14 and (useful or benefit\$1 or advantag\$4)	711	<u>L9</u>		
<u>L8</u>	15 and (benifit or advantig\$4)	1	<u>L8</u>		
<u>L7</u>	SuperScript near5 (benifit or advantig\$4)	0	<u>L7</u>		
<u>L6</u>	L5 and Notl	2	<u>L6</u>		
<u>L5</u>	SuperScript near5 reverse transcriptase\$1 near5 cDNA	127	<u>L5</u>		
<u>L4</u>	SuperScript near5 reverse transcriptase\$1	735	<u>L4</u>		
<u>L3</u>	L2	0	<u>L3</u>		
<u>L2</u>	L1 and superscript	0	<u>L2</u>		
<u>L1</u>	5968784.pn.	2	<u>L1</u>		

### END OF SEARCH HISTORY

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=> s SuperScript(10a)stud####(10a)cDNA
             0 SUPERSCRIPT(10A) STUD####(10A) CDNA
=> s SuperScript (10a)cDNA
            40 SUPERSCRIPT (10A) CDNA
T<sub>1</sub>2
=> s 12 and stud####
            13 L2 AND STUD####
L3
=> dup rem 13
PROCESSING COMPLETED FOR L3
             12 DUP REM L3 (1 DUPLICATE REMOVED)
=> d 14 1-12 bib ab kwic
     ANSWER 1 OF 12 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
L4
     2004:133932 BIOSIS
AN
     PREV200400132158
DN
     Hepcidin expression is down-regulated in alcohol-fed rats: A possible
TI
     pathogenic factor in alcohol related hepatic siderosis.
     Cheung, Ting Kin [Reprint Author]; Fletcher, Linda [Reprint Author];
ΑU
     Bridle, Kim; Murphy, Therese [Reprint Author]; Crawford, Darrell [Reprint
     Authorl
     Princess Alexandra Hospital, Woolloongabba, QLD, Australia
CS
     Hepatology, (October 2003) Vol. 38, No. 4 Suppl. 1, pp. 661A. print.
SO
     Meeting Info.: 54th Annual Meeting of the American Association for the
     Study of Liver Diseases. Boston, MA, USA. October 24-28, 2003. American
     Association for the Study of Liver Diseases.
     ISSN: 0270-9139 (ISSN print).
     Conference; (Meeting)
DT
     Conference; Abstract; (Meeting Abstract)
LΑ
     English
     Entered STN: 10 Mar 2004
ED
     Last Updated on STN: 10 Mar 2004
     Introduction: Both alcohol and excess iron in the liver can induce tissue
AB
     damage and lead to fibrosis and ultimately cirrhosis. Hepatic siderosis
     is often seen in patients with alcoholic liver disease. The pathogenic
     mechanisms underlying this phenomenon have not been well characterized.
     Hepcidin, a recently discovered liver-derived circulating peptide, has
     been implicated in the homeostasis of iron metabolism and in the
     regulation of iron absorption. Hepcidin is believed to be a negative
     regulator of intestinal iron absorption. Divalent metal transporter 1 (DMT1) and iron-regulated protein (IREG1) are the major iron transporters
     in duodenal enterocytes, with DMT1 responsible for apical uptake and IREG1
     for basolateral export of iron. The effect of alcohol on the expression
     of hepcidin, DMT1 and IREG1 is not known. Aim: To study the
     hepatic gene expression of hepcidin, DMT1 and IREG1 in a rat model of
     alcoholic liver disease. Methods: Male Sprague Dawley rats (n=10) were
     pair-fed an alcoholic (Lieber-deCarli) liquid diet or a control liquid
     diet for 12 weeks. Blood ethanol levels and serum AST were measured.
     Total liver RNA was extracted using Trizol(tm). cDNA was
     constructed using Superscript II(tm) following the
     manufacturer's instructions. Quantitative (real-time) PCR was performed
     using SYBR green and specific primers for the hepcidin gene, DMT1 and
      IREGI-with GAPDH as the control gene. Hepatic steatosis, inflammation and
      fibrosis were assessed histologically and hepatic iron concentration was
     measured colorimetrically. Results: Blood ethanol levels in alcohol-fed
      rats were significantly higher than controls (229.2+-32.1 vs. 4.5+-0.4
     mg/dL; p<0.002). Serum AST concentration was mildly increased in the
      alcohol-fed group but did not achieve statistical significance (101+-10.1
      vs. 87.8+-3.3 U/ml). Histological examination revealed extensive
      steatosis in the alcohol-fed rats with minimal inflammation and fibrosis.
      Serum ferritin (656.6+-41.2 vs. 973.2+-186.2 mug/L) and hepatic iron
      concentrations (6.2+-0.8 vs. 6.8+-0.7 mumol/g dry weight) were not
```

significantly different. Hepcidin mRNA expression was significantly decreased (7.4 fold reduction, p=0.009) in alcohol-fed rats compared to their pair-fed controls, however, hepatic DMT1 and IREG1 remained unchanged. Conclusion: Hepcidin expression is significantly decreased in the alcohol-fed rats. Altered hepcidin expression may be an important contributing factor to hepatic siderosis often seen in human alcoholic liver disease.

- . export of iron. The effect of alcohol on the expression of AB. hepcidin, DMT1 and IREG1 is not known. Aim: To study the hepatic gene expression of hepcidin, DMT1 and IREG1 in a rat model of alcoholic liver disease. Methods: Male Sprague. . . liquid diet for 12 weeks. Blood ethanol levels and serum AST were measured. Total liver RNA was extracted using Trizol(tm). cDNA was constructed using Superscript II(tm) following the manufacturer's instructions. Quantitative (real-time) PCR was performed using SYBR green and specific primers for the hepcidin gene,.
- ANSWER 2 OF 12 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN L4
- AN2003:402066 BIOSIS
- DNPREV200300402066
- Detection and partial cloning of a novel gene transcript from activated TΙ rat macrophages.
- Baker, Brian C. [Reprint Author]; Chapman, Judith; Jadus, Martin; ΑU Williams, Christopher; Klasen, Maik; Acey, Roger A.
- CS Chemistry and Biochemistry, California State University, 1250 Bellflower, Long Beach, CA, 90840, USA yadirab@juno.com; microchixx@aol.com; jadus.martin@long-beach.va.gov; stlukemd@aol.com; maikklasen@hotmail.com; racey@csulb.edu
- SO FASEB Journal, (March 2003) Vol. 17, No. 4-5, pp. Abstract No. 372.11. http://www.fasebj.org/. e-file. Meeting Info.: FASEB Meeting on Experimental Biology: Translating the Genome. San Diego, CA, USA. April 11-15, 2003. FASEB. ISSN: 0892-6638 (ISSN print).
- DT Conference; (Meeting)
  - Conference; Abstract; (Meeting Abstract)
- English LA
- ED Entered STN: 3 Sep 2003
  - Last Updated on STN: 3 Sep 2003
- AB Previous studies have shown that when T9 glioma tumor cells transfected with membrane bound macrophage colony stimulating factor (mM-CSF) are injected intra-cranially into rats, a macrophage cytotoxic response to the tumor is induced; 80% of the animals survived for at least 10 days. In addition, these animals develop an immunity to subsequent intracranial injections of parental T9 tumor cells. Our objective is to identify gene products that are induced in macrophages activated by mM-CSF. RNA from different temporal stages of MCSF activated macrophages was analyzed by differential display for ETS. Total RNA was converted to cDNA using Superscript II reverse transcriptase and anchored oligo dT as primer. The cDNA was then amplified using the same oligo dT (3'primer) and random decamers (5'primer). Analysis of the PCR products on urea-polyacryalamide gels revealed a number of differentially expressed transcripts. In one case, a prominent ETS (ca. 800 bp) is down regulated during the first 8 hours of activation. The ETS has significant homology to a Riken cDNA clone from a mouse mammary tumor. Northern blot analysis of macrophage RNA revealed the full-length transcript to be ca.
- AB Previous studies have shown that when T9 glioma tumor cells transfected with membrane bound macrophage colony stimulating factor (mM-CSF) are injected intra-cranially. . . from different temporal stages of MCSF activated macrophages was analyzed by differential display for ETS. Total RNA was converted to cDNA using Superscript II reverse transcriptase and anchored oligo dT as primer. The cDNA was then amplified using the same oligo dT (3'primer).

```
ANSWER 3 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
     2003:588647 CAPLUS
ΝA
     139:112759
DN
     Protein and cDNA sequences of a human glial-derived neurotrophic factor
TI
     and therapeutic use for nervous system diseases
     Zhou, Shoushan; Zheng, Zanshun; Fang, Haizhou; Chen, Yong; Jiang, Ruofeng;
IN
     Zhu, Aitang; Zhang, Qi; Gan, Shuyan; Lan, Xuan
     Zhuhai Yisheng Biopharmaceuticals Co., Ltd., Peop. Rep. China
PA
     Faming Zhuanli Shenqing Gongkai Shuomingshu, 28 pp.
SO
     CODEN: CNXXEV
     Patent
DΤ
     Chinese
LA
FAN.CNT 1
                                           APPLICATION NO. DATE
                    KIND DATE
     PATENT NO.
                                           _____
     CN 2001-107450 20010111
                     Α
                            20020821
     CN 1364812
                            20010111
PRAI CN 2001-107450
     The invention provides the DNA sequence or its DNA fragments and their
     encoded amino acid sequences of human glial-derived neurotrophic factor
     (GDNF) cloned from human glioma cell line C6 or synthesized by solid-phase
     synthesis method. The invention relates to the construction of the expression vector, the expression of GDNF in E.coli, yeast, and CHO cells,
     and separation and purification of GDNF from the cultured products of the above
     genetically engineered bacteria or CHO cells. The invention also relates
     to the application of the expressed GDNF in preparing the medical composition
     (composed of GDNF, natural ganglioside or its derivative, and/or mycose or
     hyaluronic acid) for treating nervous system disease, insanity, etc.
     Carbohydrates, biological studies
TT
     Gangliosides
     RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
         (protein and cDNA sequences of human glial-derived neurotrophic factor
        and therapeutic use for nervous system diseases)
     9068-38-6, Reverse transcriptase
IT
     RL: RGT (Reagent); RACT (Reactant or reagent)
         (superscript; protein and cDNA sequences of human
        glial-derived neurotrophic factor and therapeutic use for nervous
        system diseases)
     ANSWER 4 OF 12 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
L4
AN
     2003:367798 BIOSIS
     PREV200300367798
DN
     Comparison of Differentially Expressed Genes between AML and RAEBt Using
TI
     Two Different Softwares for Data Analysis.
     Bellido, Mar [Reprint Author]; Stirewalt, Derek L. [Reprint Author];
AU
     Pogosova, Era L. [Reprint Author]; Kussick, Steve [Reprint Author]; Sala,
     Olga [Reprint Author]; Deeg, Joachim [Reprint Author]; Radich, Jerald P.
      [Reprint Author]
     Clinical Research Division, Fred Hutchinson Cancer Research Center,
CS
     Seattle, WA, USA
     Blood, (November 16 2002) Vol. 100, No. 11, pp. Abstract No. 4314. print.
SO
     Meeting Info.: 44th Annual Meeting of the American Society of Hematology.
     Philadelphia, PA, USA. December 06-10, 2002. American Society of
     Hematology.
     CODEN: BLOOAW. ISSN: 0006-4971.
DT
     Conference; (Meeting)
     Conference; (Meeting Poster)
     Conference; Abstract; (Meeting Abstract)
     English
 LΆ
     Entered STN: 13 Aug 2003
 ED
     Last Updated on STN: 13 Aug 2003
     Aim : Microarray analysis using oligonucleotide arrays was performed to
 AB
      identify differentially expressed genes between de novo AML and RAEBt.
```

determine if reproducible genetic profiles can be consistently obtained,

two different softwares were used for data analysis. Methods : Eight samples from 4 patients with de novo AML and 4 patients with RAEBt were studied. The samples were processed as described in the Affymetrix GeneChip Expression Analysis Technical Manual using 5 ug of total RNA. Briefly, total RNA was converted to first-strand cDNA using Superscript II reverse transcriptase primed by an oligomer that incorporated the T7 promoter. After the synthesis of the second-strand cDNA, an in vitro transcription for linear amplification of each transcript was done using biotinylated nucleotides. The cRNA products were fragmented, heated and hybridized to the human array HG-U133A chip (Affymetrix) which contains 22577 human genes. microarrays were washed and stained and fluorescence was amplified and collected in a confocal scanner. Data analysis : The software developed for Affymetrix (M.A.S 5.0) provided the initial data to perform a global comparison between the experiments. Probe pairs were scored positive or negative following this formula: R = (PM-MM)/(PM + MM) and global scaling was applied to allow comparison of gene signals across multiple microarrays. The default discrimination threshold (tau = 0.015) was used to assign a call of absent, present or marginal for each assayed gene, and a p-value was calculated to reflect confidence in the detection call. This single array analysis generated the expression values for each gene and the file with the intensity of each probe cell (.CEL). We applied 2 different softwares, geneplus v1.2 (Enodar BioLogic Corporation, Seattle) based on regression analysis and dCHIP v1.1 (Harvard University, Boston), which uses the model-based expression approach to analyze multiple arrays. Results: Data analysis resulted in different sets of genes, 70 using geneplus and 40 using dCHIP. Proteins belonging to the zinc-finger protein family, bone marrow matrix, transcription factors and to clones with unknown genes constituted most of the differentially expressed genes. An exploratory clustering using both sets of genes allowed to differentiate AML from RAEBt. Comments : Data analysis of oligonucleotide arrays performed with different measures of transcript amount can result in different results, even although the same initial dataset is used.

. . for data analysis. Methods : Eight samples from 4 patients with de AB. novo AML and 4 patients with RAEBt were studied. The samples were processed as described in the Affymetrix GeneChip Expression Analysis Technical Manual using 5 ug of total RNA. Briefly, total RNA was converted to first-strand cDNA using Superscript II reverse transcriptase primed by an oligomer that incorporated the T7 promoter. After the synthesis of the second-strand cDNA, an.

ANSWER 5 OF 12 MEDLINE on STN L4

DUPLICATE 1

MEDLINE 2002247902 AN

PubMed ID: 11986703 DN

Expression and drug resistance of human MGMT gene in hemopoietic cells ΤI mediated by bicistronic retroviral vector.

Wang J; Xia X; Chen Z; Ruan C G UA

- Jiangsu Institute of Hematology, First Affiliated Hospital of Suzhou CS Medical College, Suzhou 215006, China.
- Zhonghua shi yan he lin chuang bing du xue za zhi = Zhonghua shiyan he SO linchuang bingduxue zazhi = Chinese journal of experimental and clinical virology, (2001 Sep) 15 (3) 265-8. Journal code: 9602873. ISSN: 1003-9279.

China

CY Journal; Article; (JOURNAL ARTICLE) DT

LΑ Chinese

Priority Journals FS

ΕM 200302

Entered STN: 20020503

Last Updated on STN: 20030227 Entered Medline: 20030226

OBJECTIVE: To increase myeloid progenitors resistance to chemotherapy and AB prevent myelosuppression caused by alkylating agents. METHODS: Total cellular RNA was extracted from human liver and cDNA was

synthesized by superscript reverse transcriptase, a polymerase chain reaction (PCR) was conducted. We obtained a full length cDNA fragment encoding human alkyguarine-DNA-alhyltransferase(MGMT). The PCR product was cloned into pGEMT-T vector and further subcloned into G1Na retrovirus expression vector. Then the recombinant plasmid was transduced into the packaging cell lines GP+E86 and PA317 by lipofect AMINE. RESULTS: By using the medium containing BCNU for cloning selection and ping-ponging supernatant infection between ecotropic produced clone and amphotropic producer clone, we obtained high titer amphotropic PA317 producer clone with the highest titer up to 8.6-10 CFU/ml. Human hematopoietic cells were infected repeatedly with this high titer virus under stimulation of hemopoietic growth factors IL-3, IL-6 and SCF. PCR, RT-PCR, Southern blot, Western blot and MTT analyses showed that MGMT gene has integrated into the genomic DNA of human hemopoietic cells and expressed efficiently. CONCLUSIONS: This study provides a foundation for application of gene therapy to tumor clinical trial. . resistance to chemotherapy and prevent myelosuppression caused by alkylating agents. METHODS: Total cellular RNA was extracted from human liver and cDNA was synthesized by superscript reverse transcriptase, a polymerase chain reaction(PCR) was conducted. We obtained a full length cDNA fragment encoding human alkyguarine-DNAalhyltransferase(MGMT). The PCR. . . analyses showed that MGMT gene has integrated into the genomic DNA of human hemopoietic cells and expressed efficiently. CONCLUSIONS: This study provides a foundation for application of gene therapy to tumor clinical trial.

- L4 ANSWER 6 OF 12 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
- AN 2001030872 EMBASE
- TI Toll-like receptor 4: The missing link of the cerebral innate immune response triggered by circulating gram-negative bacterial cell wall components.
- AU Laflamme N.; Rivest S.
- CS S. Rivest, Lab. of Molecular Endocrinology, CHUL Research Center, Laval University, 2705, boul. Laurier, Quebec, Que. GIV 4G2, Canada. Serge.Rivest@crchul.ulaval.ca
- SO FASEB Journal, (2001) 15/1 (155-163).

Refs: 19

ISSN: 0892-6638 CODEN: FAJOEC

- CY United States
- DT Journal; Article
- FS 026 Immunology, Serology and Transplantation
- LA English

AB

- SL English
- The recent characterization of human homologues of Toll may be the missing AB link for the transduction events leading to NF- $\kappa B$  activity and proinflammatory gene transcription during innate immune response. Indeed, CD14 is not thought to participate directly in the cell signaling, but rather one or more of the mammalian Toll-like receptors (TLRs) acts in concert with the lipopolysaccharide (LPS) receptor to discriminate between microbial pathogens or their products and initiate transmembrane signaling. Mammalian cells may express as many as 10 distinct TLRs, although the importance of TLR4 in response to gramnegative bacteria and LPS is now supported by the fact that TLR4-mutated mice are LPS resistant. We investigated the expression of TLR4 across the rat brain under basal conditions and in response to systemic LPS and IL-1 $\beta$  injection. We first cloned the rat TLR4 cDNA via RNA isolation and polymerase chain reaction (PCR) amplification with a proofreading polymerase. Total RNA was isolated from the rat liver tissue using Tri-Reagent and reverse transcribed into cDNA using Superscript II reverse transcriptase and an oligonucleotide primer with a degenerate 3' end of sequence 5'-T12(GAC)N-3'. Positive hybridization signal was found in the leptomeninges, choroid plexus (chp), subfornical organ, organum vasculosum of the lamina terminalis, median eminence, and area postrema. Scattered

small cells also displayed a convincing hybridization signal within the brain parenchyma. Few well-defined nuclei exhibited positive TLR4 transcript: the supramamillary nucleus, cochlear nucleus, and the lateral reticular nucleus. The circumventricular organs, the leptomeninges, and chp also exhibited constitutive expression of the LPS receptor mCD14. In contrast to the strong up-regulation of the gene encoding mCD14 during endotoxemia, neither LPS nor IL-1β caused a convincing increase in the TLR4 mRNA levels across the CNS. A down-regulation of the gene encoding TLR4 was found in the cerebral tissue of immunechallenged animals. The constitutive expression of both mCD14 and TLR4 may explain the innate immune response in the brain, which originates from the structures devoid of blood-brain barrier in presence of circulating LPS. . amplification with a proofreading polymerase. Total RNA was isolated from the rat liver tissue using Tri-Reagent and reverse transcribed into cDNA using Superscript II reverse transcriptase and an oligonucleotide primer with a degenerate 3' end of sequence 5'-T12(GAC)N-3'. Positive hybridization signal was found. Medical Descriptors: \*brain \*immunity \*bacterial cell wall \*Gram negative bacterium \*nucleotide sequence signal transduction inflammation polymerase chain reaction blood brain barrier in situ hybridization microglia septic shock cytokine release molecular cloning RNA probe nonhuman male rat animal experiment animal model controlled study animal tissue article priority journal \*toll like receptor 4: EC, endogenous compound \*immunoglobulin enhancer binding protein: EC, endogenous compound \*bacterium lipopolysaccharide: EC, endogenous compound ANSWER 7 OF 12 MEDLINE on STN 2001351183 MEDLINE PubMed ID: 11414441 A novel method for the preparation of large cDNA fragments from dengue-3 RNA genome by long RT-PCR amplification. Attatippaholkun W H; Attatippaholkun M K; Nisalak A; Vaughn D W; Innis B L Department of Clinical Chemistry, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand. Southeast Asian journal of tropical medicine and public health, (2000) 31 Suppl 1 126-33. Journal code: 0266303. ISSN: 0125-1562. Thailand Journal; Article; (JOURNAL ARTICLE) English Priority Journals 200110

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AU CS

SO

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DΤ

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Entered STN: 20011029

Last Updated on STN: 20011029 Entered Medline: 20011025

AΒ

AB

For many years, dengue viruses were among the most difficult flaviviruses to isolate and to identify, but technical advances in the past 20 years have facilitated this process. Dengue viruses are usually recovered from specimens by the infection of mosquito-cell cultures. The virus may be passaged several times in cell cultures until a sufficient infectivity titer is attained. The viral nucleocapsid consists of capsid protein and an RNA genome. The dengue genome is a single stranded messenger (positive) sense RNA of approximately 11 kb in length. The isolation of dengue genomic RNA from various sources requires precautions to avoid RNases. RNases are released during cell disruption, and their activity must be inhibited as quickly as possible by using guanidinium thiocyanate in the presence of 2-mercaptoethanol. There has recently been a revolution in molecular biology with the development of the powerful reverse transcriptase (RT) and polymerase chain reaction (PCR) technology. Advanced studies on RT technique lead to much further improvement of the reverse transcriptase enzyme by genetic engineering. The Superscript II RNase H- RT (GIBCO BRL, USA) is genetically engineered DNA polymerase that synthesizes a complementary DNA strand from single-stranded RNA. DNA or an RNA-DNA hybrid. This enzyme is produced from a cloned M-MLV RT gene constructed by the introduction of point mutation in the RNase H active center. The selective mutations within the RNase H domain maintain full polymerase activity. This structural modification eliminates degradation of RNA molecules during the first strand cDNA synthesis. The combination of thermostable DNA polymerase with and without proofreading activity (3'-exonuclease activity), improved buffer conditions and thermal cycling profiles overcome the length limitation of PCR. On the basis of these findings, we have developed a long RT-PCR system for preparing large cDNA fragments of dengue 3 virus (H-87) by using the Superscript II RNase H- RT for reverse transcription and a mixture of Taq and Pwo DNA polymerases for Three large cDNA fragments covered the full genomic RNA from the 5'-end to the 3'-end of dengue-3 virus (H-87; 10,696 bps) could be successfully prepared as the lengths of 2.437 bps, 3,980 bps and 4,337 bps respectively. The ability of our developed long RT-PCR will bring speed and simplicity to genomic mapping and sequencing and facilitate studies in molecular genetics of dengue viruses.

. . revolution in molecular biology with the development of the powerful reverse transcriptase (RT) and polymerase chain reaction (PCR) technology. Advanced studies on RT technique lead to much further improvement of the reverse transcriptase enzyme by genetic engineering. The Superscript II RNase. . . length limitation of PCR. On the basis of these findings, we have developed a long RT-PCR system for preparing large cDNA fragments of dengue 3 virus (H-87) by using the Superscript II RNase H- RT for reverse transcription and a mixture of Taq and Pwo DNA polymerases for PCR. Three large. . . respectively. The ability of our developed long RT-PCR will bring speed and simplicity to genomic mapping and sequencing and facilitate studies in molecular genetics of dengue viruses.

L4 ANSWER 8 OF 12 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

TI The effect of low sodium diet on loop of Henle bicarbonate transport and medullary thick ascending limb NHE-3 gene expression.

AU Capasso, Giovambattista [Reprint author]; Rizzo, Maria [Reprint author]; Deligatti, Angela [Reprint author]; Ferrara, Daniele [Reprint author]

CS Nephrology, Faculty of Medicine, Second University of Naples, Naples,

Journal of the American Society of Nephrology, (September, 2000) Vol. 11, No. Program and Abstract Issue, pp. 2A. print.

Meeting Info.: 33rd Annual Meeting of the American Society of Nephrology and the 2000 Renal Week. Toronto, Ontario, Canada. October 10-16, 2000.

AN 2002:223671 BIOSIS

DN PREV200200223671

American Society of Nephrology. CODEN: JASNEU. ISSN: 1046-6673.

DT Conference; (Meeting)

Conference; Abstract; (Meeting Abstract)

Conference; (Meeting Poster)

LA English

ED Entered STN: 3 Apr 2002

Last Updated on STN: 3 Apr 2002

- It has been reported that sodium intake may affect renal tubular AB acidification. Previous data have also demonstrated that most of the bicarbonate transport along the loop of Henle (LOH) is localised at the level of the medullary thick ascending limb (mTAL) and it is mainly dependent from the presence of luminal Na+-H+ exchanger (NHE-3). present study we have investigated the effect of low sodium diet (LSD) on: 1. bicarbonate transport along the LOH; 2. NHE-3 gene expression at the level of mTALs. The experiments were performed on two groups of rats: 1. control animals; 2. rats fed for fourteen days with LSD. To measure net bicarbonate reabsorption (JHCO3) superficial loops were perfused from late proximal to early distal tubules by in vivo micropuncture. Perfusate was an end-like proximal solution containing 3H-methoxy-inulin. Total (CO2) in the collected fluid was measured by microcalorimetry. In the presence of similar LOH perfusion rate (39.1+-0.9 vs 38.7+-0.9 nl/min), JHCO3 was 302+-26 pmol/min (n=20) (mean+-ES) in control animals and 295+-23 pmol/min (n=22, p>0.05) in rats under LSD. For the molecular biology experiments total RNA was extracted from mTALs, identified and microdissected from collagenase treated kidneys. cDNA was synthesised from RNA using SuperScript RT Rnase, H-reverse Transcriptase and oligo(dT) 12-18 primers; each reaction was performed in parallel with an otherwise identical reaction that contained no Reverse Transcriptase. NHE-3 mRNA abundance was quantified by a competitive PCR using an internal standard of cDNA which differed from the wild-type NHE-3 by a deletion of 76 bp. The PCR was performed starting from the same amount of total RNA. NHE-3 mRNA abundance was 0.133+-0.016 amol/ng total RNA in controls (n=5) and it decreased to 0.068+-0.007 amol/ng total RNA in rats under LSD (n=5, These data demonstrate that LSD does not affect LOH bicarbonate transport, but it decreases NHE-3 gene expression along mTAL. Therefore the preservation of unchanged proton secretion along the mTAL may be dependent from other molecular mechanism(s).
- AB. . . thick ascending limb (mTAL) and it is mainly dependent from the presence of luminal Na+-H+ exchanger (NHE-3). In the present study we have investigated the effect of low sodium diet (LSD) on:
  1. bicarbonate transport along the LOH; 2. NHE-3 gene. . . under LSD. For the molecular biology experiments total RNA was extracted from mTALs, identified and microdissected from collagenase treated kidneys.

  cDNA was synthesised from RNA using SuperScript RT

  Rnase, H-reverse Transcriptase and oligo(dT) 12-18 primers; each reaction was performed in parallel with an otherwise identical reaction that. .
- L4 ANSWER 9 OF 12 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
- AN 2001:87416 BIOSIS

ISSN: 0190-5295.

- DN PREV200100087416
- TI Toll-like receptor 4: the missing link of the cerebral innate immune response triggered by circulating Gram-negative bacterial cell wall components.
- AU Laflamme, N. [Reprint author]; Rivest, S.
- CS CHUL Research Center, Sainte-Foy, PQ, Canada
- So Society for Neuroscience Abstracts, (2000) Vol. 26, No. 1-2, pp. Abstract No.-242.17. print.

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The recent characterization of human homologues of Toll may be the missing AB link for the transduction events leading to NF-kappaB activity and proinflammatory gene transcription during innate immune response. Indeed, CD14 is not thought to participate directly in the cell signaling, but rather one or more of the mammalian Toll-like receptors (TLRs) acts in concert with the LPS receptor to discriminate between microbial pathogens or their products and initiate transmembrane signaling. Mammalian cells may express as many as ten distinct TLRs, although the importance of TLR4 in response to Gram-negative bacteria and LPS is now supported by the fact that TLR4-mutated mice are LPS resistant. The present study investigated the expression of TLR4 across the rat brain under basal conditions and in response to systemic LPS and IL-1beta injection. We first cloned the rat TLR4 cDNA via RNA isolation and PCR amplification with a proof-reading polymerase. Total RNA was isolated from rat liver tissue using Tri-Reagent and reverse transcribed into cDNA using Superscript II reverse transcriptase and an oligonucleotide primer with a degenerate 3' end of sequence 5'-T12(GAC)N-3'. Positive hybridization signal was found in the leptomeninges, choroid plexus (chp), subfornical organ, organum vasculosum of the lamina terminalis, median eminence and area postrema. Scattered small cells also displayed a convincing hybridization signal within the brain parenchyma, although the intensity was quite variable among animals. Few neuronal structures exhibited positive TLR4 transcript, namely the supramammillary nucleus, cochlear nucleus and caudal ventrolateral medulla. Of interest is the fact the circumventricular organs, the leptomeninges and chp also exhibited constitutive expression of the LPS receptor mCD14. In contrast to the strong upregulation of the gene encoding mCD14 during endotoxemia, neither LPS nor IL-1beta caused a convincing increase in the TLR4 mRNA levels across the CNS. The constitutive expression of both mCD14 and TLR4 may explain the innate immune response in the brain, which originates from the structures devoid of blood brain barrier in presence of circulating LPS.

AB. . . response to Gram-negative bacteria and LPS is now supported by the fact that TLR4-mutated mice are LPS resistant. The present study investigated the expression of TLR4 across the rat brain under basal conditions and in response to systemic LPS and IL-1beta. . . PCR amplification with a proof-reading polymerase. Total RNA was isolated from rat liver tissue using Tri-Reagent and reverse transcribed into cDNA using Superscript II reverse transcriptase and an oligonucleotide primer with a degenerate 3' end of sequence 5'-T12(GAC)N-3'. Positive hybridization signal was found. . .

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L4 ANSWER 10 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
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AN 1998:761906 CAPLUS

DN 130:11283

TI Production and purification of nucleic acid molecules using affinity-labeled primer-adapter molecules

IN Gruber, Christian E.; Jessee, Joel A.

PA Life Technologies, Inc., USA

SO PCT Int. Appl., 50 pp. CODEN: PIXXD2

DT Patent

LA English

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PI

PATENT NO. KIND DATE APPLICATION NO. DATE

WO 9851699 A1 19981119 WO 1998-US9586 19980512

W: JP

RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

EP 981535 A1 20000301 EP 1998-922202 19980512

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI

JP 1998-549380 19980512

JP 2002502237 T2 20020122 PRAI US 1997-46219P P 19970512

WO 1998-US9586 W 19980512

The present invention is directed to methods for the production and isolation of nucleic acid mols. In particular, the invention concerns isolation of mRNA mols. and the production and isolation of nucleic acid mols. (e.g., cDNA mols. or libraries), which may be single- or double-stranded. Addnl., the invention concerns selection and isolation of particular nucleic acid mols. of interest from a sample which may contain a population of mols. Specifically, the invention concerns affinity-labeled primer-adapter mols. which allow improved isolation and production of such nucleic acid mols., increasing both product recovery and speed of isolation. A mRNA or poly(A) + RNA mols is mixed with a polymerase and/or reverse transcriptase and a primer-adapter nucleic acid mol., wherein the primer-adaptor comprises one or more ligand mols. and one or more cleavage sites (for endonuclease or restriction endonuclease). The primer-adaptor may be designed to hybridize to any portion of the template. Upon incubation under appropriate conditions, a first nucleic acid mol. (e.g., a single-stranded cDNA) complementary to all or a portion of the template is made, which contains the primer-adaptor and thereby facilitates isolation of the first nucleic acid mol. and/or any nucleic acid mol. hybridized to the first nucleic acid. Such isolation may be accomplished by ligand-hapten interactions, where the hapten is bound to a solid support. Multiple synthesis with primer-adaptor mols. may result in a synthesized nucleic acid mol. having more than one primer-adaptor. The present invention is particularly suited for the rapid production and isolation of cDNA libraries from small amts. (as little of 5 ng) of input poly(A) + RNA or mRNA in a high-throughput manner. About 3-4-fold greater yield of cDNA is achieved by the present invention than with the SUPERSCRIPT Plasmid System, with approx. equivalent transformation efficiencies and average insert sizes.

RE.CNT 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT

The present invention is directed to methods for the production and isolation AB of nucleic acid mols. In particular, the invention concerns isolation of mRNA mols. and the production and isolation of nucleic acid mols. (e.g., cDNA mols. or libraries), which may be single- or double-stranded. Addnl., the invention concerns selection and isolation of particular nucleic acid mols. of interest from a sample which may contain a population of mols. Specifically, the invention concerns affinity-labeled primer-adapter mols. which allow improved isolation and production of such nucleic acid mols., increasing both product recovery and speed of isolation. A mRNA or poly(A) + RNA mols is mixed with a polymerase and/or reverse transcriptase and a primer-adapter nucleic acid mol., wherein the primer-adaptor comprises one or more ligand mols. and one or more cleavage sites (for endonuclease or restriction endonuclease). The primer-adaptor may be designed to hybridize to any portion of the template. Upon incubation under appropriate conditions, a first nucleic acid mol. (e.g., a single-stranded cDNA) complementary to all or a portion of the template is made, which contains the primer-adaptor and thereby facilitates isolation of the first nucleic acid mol. and/or any nucleic acid mol. hybridized to the first nucleic acid. Such isolation may be accomplished by ligand-hapten interactions, where the hapten is bound to a solid support. Multiple synthesis with primer-adaptor mols. may result in a synthesized nucleic acid mol. having more than one primer-adaptor. The present invention is particularly suited for the rapid production and isolation of cDNA libraries from small amts. (as little of 5 ng) of input poly(A) + RNA or mRNA in a high-throughput manner. About 3-4-fold greater yield of cDNA is achieved by the present invention than with the SUPERSCRIPT Plasmid System, with approx. equivalent transformation efficiencies and average insert sizes.

Antibodies Antigens Avidins CD4 (antigen) Collagens, biological studies Colony stimulating factor receptors Cytokine receptors Cytokines Enzymes, biological studies Fibrinogens Fibronectins Glycophorins Growth factors, animal Immunoglobulin receptors Insulin receptors Interleukins LFA-1 (antigen) Laminins Lipopolysaccharides Spectrins Transferrin receptors Transferrins Vitronectin RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (ligand mol. for affinity labeling; production and purification of nucleic acid mols. using affinity-labeled primer-adapter mols.) 1404-26-8, Polymyxin B 7439-89-6, Iron, biological 58-85-5, Biotin IT 9001-29-0, Blood-coagulation factor X 9004-10-8, Insulin, biological studies 9013-20-1, Streptavidin 62683-29-8, Colony-stimulating 20074-52-6, biological studies 80804-53-1, Complement C3bi RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses) (ligand mol. for affinity labeling; production and purification of nucleic acid mols. using affinity-labeled primer-adapter mols.) ANSWER 11 OF 12 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. L4 on STN 1998127821 EMBASE AN A competitive reverse transcription-PCR to study apolipoprotein TТ ε gene expression. Rexin M.; Feussner G. ΙIΑ G. Feussner, Med. Universitatsklinik Heidelberg, Abteilung Inhere Medizin CS I, Endokrinologie und Stoffwechsel, Bergheimer Strasse 58, D-69115 Heidelberg, Germany. uk691jd@genius.embnet.dkfz-heidelberg.de SO Clinical Chemistry, (1998) 44/4 (773-778). Refs: 31 ISSN: 0009-9147 CODEN: CLCHAU CY United States Journal; Article DTHuman Genetics FS 022 Biophysics, Bioengineering and Medical Instrumentation 027 029 Clinical Biochemistry LA English English SLWe developed a rapid and simple competitive reverse transcriptionpolymerase chain reaction for the quantification of apoarepsilon mRNA in human monocyte-derived macrophages. The method was applied, and its reliability was shown in patients with the familial lipoprotein disorder, type III hyperlipoproteinemia. Type III hyperlipoproteinemic patients express markedly higher concentrations of apos mRNA when compared

with healthy controls. Patients with this disease are usually (>90%) homozygous for a receptor binding-defective isoform of apolipoprotein apo E (apo E2). The higher expression of apos mRNA in the patients could, therefore, be a physiological mechanism to compensate for functionally defective apo E. The developed procedure might be valuable in assessment of apos gene expression in human disease. A competitive reverse transcription-PCR to study apolipoprotein ε gene expression. Medical Descriptors: \*hyperlipoproteinemia: DI, diagnosis reversed phase high performance liquid chromatography gene expression reliability receptor binding human male female clinical article controlled study human cell adult article \*apolipoprotein e: EC, endogenous compound messenger rna: EC, endogenous compound (1) Superscript Preamplification System for First Strand cDNA Synthesis; (2) Minicycler MEDLINE on STN ANSWER 12 OF 12 97193806 MEDLINE PubMed ID: 9041391 A one-tube method of reverse transcription-PCR to efficiently amplify a 3-kilobase region from the RNA polymerase gene to the poly(A) tail of small round-structured viruses (Norwalk-like viruses). Ando T; Monroe S S; Noel J S; Glass R I com du reference Viral Gastroenteritis Section, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333, USA. txa5@CIDDVD1.em.cdc.gov Journal of clinical microbiology, (1997 Mar) 35 (3) 570-7. Journal code: 7505564. ISSN: 0095-1137. United States Journal; Article; (JOURNAL ARTICLE) English Priority Journals 199706 Entered STN: 19970612 Last Updated on STN: 19980206 Entered Medline: 19970602 Amplification of a 3-kb genome region from the RNA polymerase gene to the 3' poly(A) tail of small round-structured virus (SRSV) by reverse transcription-PCR (RT-PCR) has been difficult to achieve because of a stable secondary structure in a region between the RNA polymerase gene and the 5' end of the second open reading frame. We have developed a one-tube RT-PCR method to efficiently amplify this region. The method comprises three procedures: purification of poly(A) + RNA from a starting RNA solution by oligo(dT)30 covalently linked to latex particles, buffer exchange, and continuous RT and PCR in a single tube containing all

reaction components. The key elements of this method are (i) first-strand

H- Moloney murine leukemia virus reverse transcriptase at 50 degrees C for 10 min by using the RNA-oligo(dT)30 hybrid on the latex particles as the template and primer, and (ii) PCR by Taq and Pwo DNA polymerases mixed together with a mixture of 12 phased oligo(dT)25 antisense primers. The detection threshold of the one-tube RT-PCR method was as little as 0.2 ng of the crude RNA used as the source of the template. Using this method,

cDNA synthesis with the Superscript II version of RNase

CT

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we obtained 3-kb products from 24 SRSV strains previously characterized into four genetic groups. These included 5 P1-A, 4 P1-B, 5 P2-A, and 10 P2-B strains. Because SRSVs have not yet been cultivated in vitro, this novel method should facilitate molecular characterization of SRSVs to provide a firm scientific foundation for improvements and refinements of SRSV diagnostics.

. . . RT and PCR in a single tube containing all reaction components. The key elements of this method are (i) first-strand cDNA synthesis with the Superscript II version of RNase H- Moloney murine leukemia virus reverse transcriptase at 50 degrees C for 10 min by using. . .

 $\mathtt{CT}$  . . . Human

AB

Base Sequence

Caliciviridae Infections: DI, diagnosis Caliciviridae Infections: VI, virology

DNA Primers: GE, genetics

\*DNA-Directed RNA Polymerases: GE, genetics

Evaluation Studies

Gastroenteritis: DI, diagnosis Gastroenteritis: VI, virology

\*Genes, Viral

Moloney murine leukemia virus: EN, enzymology

Norwalk virus: EN, enzymology